

Easy-to-Screen™
DNA Pools:
BAC Mouse ES
(Release I)
Product Manual



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About Incyte Genomics, Inc.

Incyte Genomics, Inc. is a leading provider of an integrated platform of genomic technologies designed to aid in the understanding of the molecular basis of disease.

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1 Overview

Incyte Genomics' Easy-to-Screen™ DNA Pools: BAC Mouse ES (Release I) are arrayed in fifteen 96-well plates. Enough DNA is supplied for you to identify clones from the primary pools using 25 or 50 different primer pairs (depending on the kit size ordered). You may need to order additional plates from the secondary plate pools and the tertiary plate pools if you have more positives from the same primary pools than is statistically probable.

Three rounds of PCR experiments are required to identify the complete clone address of your clones. The BAC Mouse ES (Release I) library represents threefold genomic coverage. Therefore, you should expect, on average, three clones from each primary pool screening. Once you have identified the clone plate and well address in the library, contact Incyte Genomics to request a stab culture of the clone.

Note: Store this kit -20°C.

Table 1-1. Construction Details for the BAC Mouse ES Release I and Release II Libraries

Library Details	BAC Mouse ES (Release I)	BAC Mouse ES (Release II)
Strain	129/SvJ	129/SvJ
Host	DH10B	DH10B
Vector	pBeloBAC11	pBeloBAC11
Cloning Site	Hind III	Hind III
Average Insert Size	120 kb	120 kb
Number of Clones	92,160	92,160
Library Plate Numbers	1–240	241–480
Cell Line/Tissue	RW4 Cells	RW4 Cells
Genomic Coverage	3.2x	3.2x
Creator	Incyte Genomics (formerly Genome Systems)	Incyte Genomics (formerly Genome Systems)

2 Experimental Protocol

The Primary Pools

The BAC Mouse ES (Release I) library is housed in 240 multi-well plates, numbered 1–240 accordingly. Incyte Genomics has prepared DNA from each plate (384 clones) individually and combined them into blocks of 10 to create the primary assay pools. This step narrows the search to a group of 10 plates.

- Primary pool 1 contains DNA from library plates 1–10.
- Primary pool 2 contains DNA from library plates 11–20.

This pattern continues through primary pool 24. Table 2-1 illustrates the pooling strategy.

Table 2-1. Primary Pools and Their Corresponding Secondary Pools

1. 1–10	9. 81–90	17. 161–170
2. 11–20	10. 91–100	18. 171–180
3. 21–30	11. 101–110	19. 181–190
4. 31–40	12. 111–120	20. 191–200
5. 41–50	13. 121–130	21. 201–210
6. 51–60	14. 131–140	22. 211–220
7. 61–70	15. 141–150	23. 221–230
8. 71–80	16. 151–160	24. 231–240

In your kit, the 24 primary pools of DNA are located in plate 1 (see Appendix A).

- Column 1 contains primary pools 1–8
- Column 2 contains primary pools 9–16
- Column 3 contains primary pools 17–24

Preliminary Experiment

We recommend that you perform a preliminary experiment with mouse genomic DNA as a PCR template to precisely determine the annealing temperature of your oligonucleotides.

Note: For those labs that work with cDNA, we recommend that you aliquot your oligos in another lab using the other lab's pipettes and pipette tips. The contamination of pools and oligos is difficult to prevent. We also recommend you use cotton-plugged pipette tips.

If the PCR product shows extraneous artifact bands, you may find a “hot start” or “touch down” program useful. These modifications may reduce artifacts generated from false priming on the genomic DNA.

See Appendix C for Incyte Genomics' in-house PCR cycle programs and reaction mixtures.

Perform two negative controls

1. A water control, and
2. An unrelated DNA control.

Note: Both of these controls must be negative before you begin the experiment.

Experiment

The primary pool screen will require 24 reactions, not including your positive genomic and negative controls.

1. When you are ready to screen, thaw DNA at room temperature.
2. Spin briefly to pull any excess DNA from the lid. Remove cap mats carefully.
3. For each PCR, use only 2 μ l of solution from each well. (Replace cap mats in their original orientation to avoid cross-well contamination.)
4. Perform 24 PCR reactions in addition to your positive and negative controls, and assay the reaction products on a 2% agarose gel with appropriate standards. See Appendix C for the standard PCR components.

Note: Unless there is a polymorphism in your PCR amplicon, the band migration in the gel should be identical to your genomic control.

5. Document your results and record the pools that have a positive result.
6. Refer to Table 2-1 on page 3. Identify the secondary pools that correspond to the primary pool that was positive by PCR.

Example:

A “hit” in primary pool 15 means that your clone is in one of the library plates 141–150. A PCR assay of each one of these individual secondary pools will tell you which of these 10 plates houses your clone.

The Secondary Pools

So far, you have identified which secondary pools need to be screened (from the primary screen and Table 2-1). Next, check Appendix A to locate the 96-well plate containing the appropriate secondary pools for the next experiment. To continue with our example, secondary pools 141–150 are contained in plates 2 and 3 of this kit.

Experiment

1. Allow DNA solutions to thaw at room temperature.
2. Spin briefly to pull any excess DNA from the lid. Remove cap mats carefully.
3. For each PCR, use only 10 μ l of solution from each well. (Replace cap mats in their original orientation to avoid cross-well contamination.) Perform the PCR in a 25 μ l reaction volume.
4. Perform PCR for 35 cycles and assay the reaction products on a 2% agarose gel with appropriate standards.

Note: Unless there is a polymorphism in your PCR amplicon, the band migration in the gel should be identical to your genomic control.

5. Document your results and record the pool that has a positive result. You have now identified the library plate that houses your clone.

For our example, let's say the positive hit is from secondary pool 142. Therefore, your clone lies in library plate 142, and you will now proceed to the tertiary pools to identify the well location within that plate.

The Tertiary Pools

One experiment remains. The primary pools narrowed your search (for our example, primary pool 15) and the secondary pools gave you the library plate number (for our example, library plate 142). You must now identify the column and row position for the well that actually houses your clone. Incyte Genomics has pooled each plate in a three-dimensional matrix to allow you to identify the well location.

Note: Refer to Appendix B to determine which 96-well kit plate should be used to determine the well that houses your clone. For our example, you would test kit plate 11, rows 1–5.

Construction of Tertiary Pools

A 384-well library plate includes 24 vertical columns (1–24) and 16 horizontal rows (a–p). Each column is pooled top to bottom such that each column pool contains 16 individual clones. Each row is pooled left to right such that each row pool contains 24 individual clones. Each plate is thus pooled into 40 separate tubes (16+24). The pooling strategy of each plate is identical. These tubes are combined into blocks of 10 to create the tertiary pools.

As with the primary pools, there are 24 groups of tertiary DNA pools. There are 40 DNA pools in each group (24 column pools and 16 row pools).

Refer to Appendix B for the location of the appropriate tertiary DNA pools. This chart will tell you which 96-well plate holds the DNA and where in that plate the DNA can be found.

Experiment

1. Allow DNA solutions to thaw at room temperature.
2. Spin briefly to pull any excess DNA from the lid. Remove cap mats carefully.

3. For each PCR, use only 10 µl of solution from each well. (Be sure to replace cap mats in the same orientation as they were to avoid cross-well contamination.) Perform the PCR in a 25 µl reaction volume.
4. Perform 40 PCR reactions in addition to your positive and negative controls, and assay the reaction products on a 2% agarose gel with appropriate standards.

Note: Unless you are aware of a polymorphism in your PCR amplicon, the band migration in the gel should be identical to your genomic control.

5. Document your results and record the pools that have a positive result. You should obtain two pools that are positive, one derived from a column pool (1–24) and the other derived from a row pool (a–p) to give the exact location of your clone.

To continue with our example, let's say the identified positives are “B” and “9”. This means that your clone is located in plate 142, well B9 in the library.

Placing a Clone Order

You may purchase the positive clone(s) by faxing an order to 1-888-919-3324 or 1-314-427-3324. You may also send e-mail orders to sales@incyte.com, or order directly through our web site at www.incyte.com/bioreagents.

Include the following information with your order:

- Name of the library (i.e., BAC Mouse ES (Release I))
- Plate and well position of each clone identified with this kit (in our example, plate 142, well B9)

3 Frequently Asked Questions

How should I store the kit?

Store the kit at -20°C.

Can I use less DNA than is suggested?

The volume given in this manual is our recommendation. This amount has repeatedly produced a robust signal. If you use less, the signal may be reduced and/or disappear.

Why is the volume of the secondary pools and tertiary pools not the same as the primary pools?

The kits are produced to offer multiple screenings. You are supplied with enough of the primary pools to do either 25 or 50 reactions (depending on the kit size you ordered). The volume of secondary pools and tertiary pools is equivalent to the probable number of times each pool should be positive. We estimate three times. To reduce the chance of complete evaporation of a small volume, we have diluted the DNA in the secondary and tertiary pools more than the primary pools.

I'm hitting more than three primary pools. What could be the cause?

On average, this library should provide three clones per screen, although it's possible that fewer or more hits can result, depending on how often the sequence is represented in the library. We generally recommend following the stronger pools only. If five or more primary pools are comparable in intensity to the genomic positive control, it is possible that your primer pair amplifies a region that is not specific for your gene of interest, and pseudogenes or homologues could be identified as well. It is also possible that the amplified area contains some repetitive sequence. If all primary pools are positive and your negative control is negative, it's likely that your primers amplify a region that is similar to the library vector sequence. For any of these cases, we recommend that you work with a different primer pair from a more suitable region of your sequence. If all primary pools are

positive and your negative control is also positive, then your primers are probably contaminated, and you will need to make new stocks.

I'm hitting a single pool in the primary and secondary pools, but when I follow through to the tertiary round, instead of getting one alphabetical pool and one numerical pool as expected, I'm hitting two of each. Does this mean that there are two clones?

It is possible that your primers will identify two clones that are in the same library plate. It is also possible that there is cross-contamination in your tertiary pool plate. If you wish to purchase all of the possible clones from a particular plate, we charge full price for the first clone from that plate and half price on any additional clones from that plate. The clones must be purchased at the same time to receive the discounted price on your order. Once you receive the clones, perform PCR for each bacterial stock to be sure that it is truly positive and to rule out the possibility of contamination in the kit plate. Please call our Technical Support staff if you have questions.

I'm hitting a single pool in the primary pools and then see two positive secondary pools and 4 positive tertiary pools (two alphabetical and two numerical hits). Since there are 8 possible combinations, how do I distinguish the real plate and well location of my clone(s)?

Example: Primary pool hit: 3

Secondary pool hits: 21 and 27

Tertiary pool hits: 1, 7, A, H

Possible plate/well combinations: 21a1, 21a7, 21h1, 21h7, 27a1, 27a7, 27h1, 27h7

On occasion, we find that two clones are found in the same group of 10 plates that were superpooled together, so one would see the single pool hit in the primary pools and then two positive pools among the secondary pools. Because the tertiary pools consist of column and row pools that were also superpooled into groups of 10 plates, the same tertiary pools will be used for identification of the well position for the two potential clones. (Remember, the tertiary pools to be used are designated by the primary pool hit, as in Appendix B.) This leads to two lettered hits and two numbered hits, creating the eight possible plate and well combinations. As we understand that this phenomenon can occur, we

offer you two options. One option is to purchase the eight potential clones at a discounted rate. The second option is to send us an aliquot of your primers to test the eight potential plate and well locations and identify the two real ones. We will give this information to you so that you can order the two clones. Please feel free to call our Technical Support staff to discuss both options.

A Key to All 96-Well Plates (1–15)

Figure A-1. Plate 1: Primary Pools and Secondary Pools 1–48

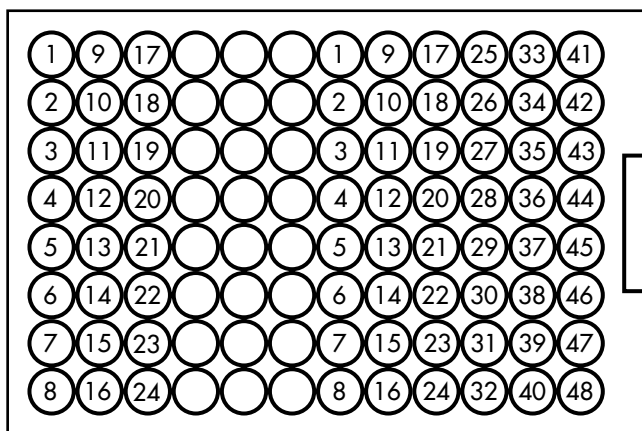


Figure A-2. Plate 2: Secondary Pools 49–144

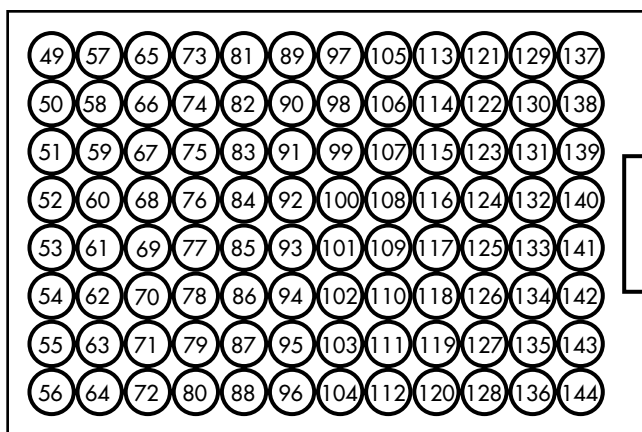


Figure A-3. Plate 3:
Secondary Pools 145–240

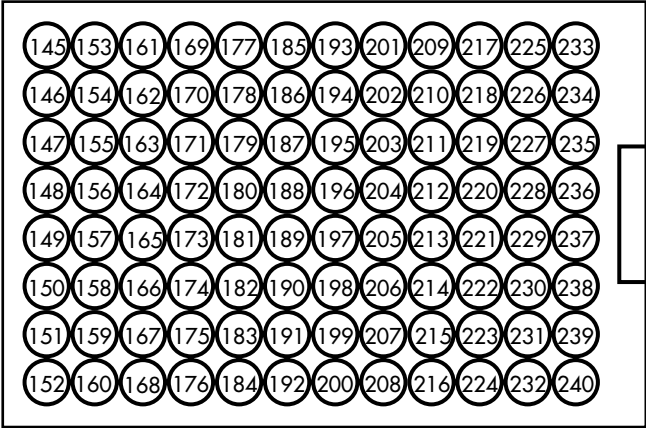
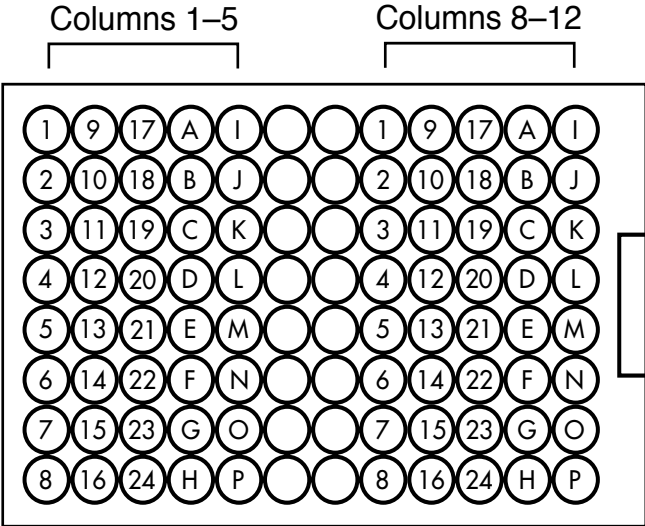


Figure A-4. Plates 4–15:
Tertiary Pools



B Key to 96-Well Plates 4–15 (Tertiary Pools)

Table B-1. Key to 96-Well Plates 4–15

Primary Pool Hit	96-Well Plate No.	Location	
		Columns 1–5	Columns 8–12
1	4	X	
2			X
3	5	X	
4			X
5	6	X	
6			X
7	7	X	
8			X
9	8	X	
10			X
11	9	X	
12			X
13	10	X	
14			X
15	11	X	
16			X
17	12	X	
18			X
19	13	X	
20			X
21	14	X	
22			X
23	15	X	
24			X

C Standard PCR Components

This appendix lists the requirements for running Incyte Genomics standard PCR experiments. All amounts listed are per reaction. The requirements include:

- Standard PCR Reaction Mixture
- 10x PCR Buffer Recipe
- Our Standard Thermocycler Program

Standard PCR Reaction Mixture

Water	*
7.5 mM dNTPs	2.5 µl
10x PCR Buffer	2.5 µl
DMSO	2.5 µl
1x Tween	2.5 µl
25 µM Primer 1	0.5 µl
25 µM Primer 2	0.5 µl
DNA	*
TAQ Polymerase	0.125 µl
<hr/>	
Total	25.0 µl

*The amount of DNA and water are variable.

10x PCR Buffer Recipe

To make 300 ml of the 10x PCR buffer, add the amounts of the components listed in Table C-1 to an appropriate container.

Table C-1. 10x PCR Buffer Recipe

Component	Amount to Add	Final Concentration
1 M MgCl ₂	8.1 ml	27 mM
1.6 M (NH ₄) ₂ SO ₄	31.125 ml	166 mM
14.1 M 2-mercaptoethanol	600 µl	28 mM
0.5 M EDTA, pH 8.0	40 µl	68 µM
1 M Tris-Cl, pH 8.8	201 ml	670 mM
dH ₂ O	59.175 ml	

Our Standard Thermocycler Program

1. 94°C for 2 min, 1 cycle
2. 94°C for 1 min
3. 45–65°C for 1 min
4. 68°C for 1 min
5. Go to step 2, repeat 34 times (35 cycles in all)
6. 68°C for 7 min
7. 4°C indefinitely